Sakarya University Journal of Science

ISSN : 2147-835X
Publisher : Sakarya University

Vol. 28, No. 4, 804-815, 2024
DOI: https://doi.org/10.16984/saufenbilder.1456568

Research Article

Investigation of Permeability of Thiocolchicoside Through Transdermal Drug Delivery System Using Franz Diffusion Cell

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ARTICLE INFO

Keywords:
Thiocolchicoside
Franz diffusion cell
Synthetic membranes
Transdermal drug delivery system
HPLC

Article History:
Received: 21.03.2024
Accepted: 22.05.2024
Online Available: 01.08.2024

ABSTRACT

Recently, drug release applications through the skin have become very popular. One of the most remarkable of these drug release applications is transdermal drug release systems, which are drug release methods that allow the active substance to pass into the systemic circulation through the skin or artificial membranes. In this study, the optimization conditions required for the release and permeation tests of a gel drug containing the active substance thiocolchicoside were comparatively investigated using synthetic membranes without human or animal skin. For this purpose, the permeability of the gel drug in gel form and the active ingredient thiocolchicoside was carried out using Franz Diffusion Cell. As a result of the investigations, it was observed that the best synthetic membrane for the permeability of thiocolchicoside in the Franz Diffusion Cell was the Supor membrane. In addition, the method's relative standard deviation values, detection, and quantification limits were determined, and permeation studies were carried out. In this study, the correlation coefficient was found to be 0.9992, and the limits of detection and quantification were 0.026 and 0.078 µg/L. In this way, the sensitivity and reliability of the validation study were determined.

1. Introduction

Colchicoside is a glucoside found in the plant *Colchicum autumnale*. A semisynthetic sulfur derivative of colchicoside is called thiocolchicoside. Thiocolchicoside acts as a potent muscle relaxant by activating the GABA-nergic inhibitory pathways, which modify muscular contracture due to its particular affinity for -amino butyric acid (GABA) receptors (Figure 1). In clinical settings, the substance is used to treat neurological, rheumatic, muscular, and traumatic problems. In animal models, anti-inflammatory and analgesic effects have also been documented. Because of the hepatic first-pass effect and limited gastrointestinal absorption, the oral bioavailability of the medication is approximately 25%. Since its low oral bioavailability limits its use, a different delivery method is preferred to achieve high blood concentrations for treatment management [1-8].

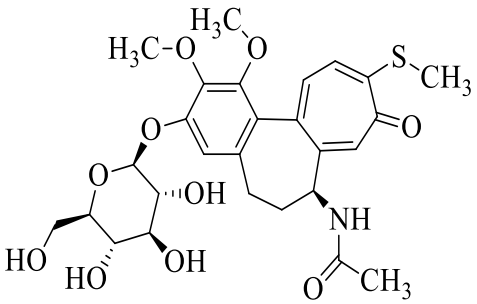

Figure 1. Chemical structure of thiocolchicoside

A transdermal drug delivery system (TDDS) is an effective system that provides controlled active drug delivery and is an alternative to

Cite as: H. Karagüzel, S. Sivrikaya Özak, A. Dalmaz (2024). Investigation of Permeability of Thiocolchicoside Through Transdermal Drug Delivery System Using Franz Diffusion Cell, *Sakarya University Journal of Science*, 28(4), 804-815. https://doi.org/10.16984/saufenbilder.1456568

traditional drug delivery method treatments. TDDS ensures that the drug applied to the skin has a therapeutic effect and that the concentration of the active ingredient passes into the bloodstream systematically and is controlled. Essential advantages of TDDS: The aim is to reduce the side effects of the drug by avoiding the first pass effect in the liver, reducing the frequency of drug administration, and ensuring a constant and stable plasma level [9-13].

Thanks to these advantages, the development of TDDS has led to its approval by regulatory authorities for its increasing importance and commercialization in the pharmaceutical industry [14-18]. To observe and test the therapeutic efficacy of TDSS, in vitro, technical, and model systems that can measure drug release permeability and obtain reliable, reproducible, validated, and accurate predictive data are required [19-24]. In addition, the applicability of TDDS is made by in vitro tests on drugs in finished dosage form. The drug's performance can be tested with a permeation test study combined with an in vitro-in vivo [25-28].

Clinical trials for TDDS offer substantial chances to save time and money on development. Scale-up in drug production and extensive changes in the performance of approved products (e.g., changes in drug supplier, formulation and manufacturer, or raw materials. Comprehensive studies by companies should ensure reproducibility [29]. In the literature on in vitro–in vivo correlations (IVIVCs) for TDDS, the recommended drug by international guidelines formulation studies in topical and transdermal dosage forms are advised to measure release and penetration [30-37]. IVIVCs, Franz-type (static) diffusion cells with two compartments, are the most widely used technical (modifiable) diffusion cells. In the Franz Diffusion Cell test, a membrane that separates the donor and the receptor and can represent human skin is needed, and synthetic membrane studies are gaining importance for this purpose [30, 38, 39].

Synthetic membranes are frequently used in drug release and permeability studies on human or animal skin [31]. Synthetic membranes are the best topical in vitro model reported to date to predict skin status in Franz cells in vivo penetration of products [40]. Regulatory authorities highly recommend them. However, the use of human skin and slaughtered animal skin is considered reasonable and is subject to national and international ethical guidelines [9, 41, 42]. Several studies have shown that pig ears can be used for testing because they have biochemical properties and structural similarities to human skin, and the ability to obtain results comparable to human skin has made them ideal for permeability studies [31, 43, 44].

Since biological skin is difficult to find and has different properties in each sample, an inexpensive and reproducible membrane model that mimics the skin barrier in terms of release and permeability is becoming very popular. In this study, the necessary optimization conditions for drug release and permeation tests were investigated by using synthetic membranes that do not contain human or animal skin. The effects of parameters such as membrane selection, membrane saturation, mixing time, mixing speed, and temperature on the permeability of the developed gel drug were investigated, and their similarity with the reference drug was proven. Fourier transform infrared spectroscopy and Differential scanning calorimetry analyses were performed to show the similarity between the developed gel drug and the reference drug.

2. Materials and Methods

2.1. Reagents and chemicals

All reagents used are of analytical purity. Potassium monobasic phosphate (KH_2PO_4), potassium chloride (KCl), sodium chloride (NaCl), disodium hydrogen phosphate (Na_2HPO_4), trifluoroacetic acid, high-performance liquid chromatography (HPLC) quality acetonitrile and methanol Merck quality 96% to 99.7%. It has varying degrees of purity. 0.45 mm polytetrafluoroethylene (PTFE) filters were used to filtrate the solutions (Millex Millipore, Istanbul, Türkiye). Ultrapure water was obtained using the Sartorius water purification (Sartorius Arium, USA). Thiocolchicoside standard (Batch No: M062300088) was purchased from INDIA GLYCOLS and used in working studies. The commercially available reference drug in gel

form containing the active ingredient thiocolchicoside was obtained from a pharmacy in Duzce (Türkiye). A drug in gel form with a new formulation was developed in the laboratory environment with the purchased thiocolchicoside standard. As a commercial membrane, PALL Supor 450 membrane disc filter 0.45µM 47 mm, plain (REF:60173), PALL HT-450 Tuffryn 100/PK Membrane Filter 0.45µM 47 m (Ref: T42575), Sartorius regenerated cellulose (RC) 0.45 µM (Ref: 1122 18406 2201853) filters were used in Franz diffusion cell experiments.

2.2. Instruments

The study used Hanson Research MicroettePlus brand Franz cell 10-1522, serial number Franz Diffusion Cell, for in vitro permeation experiments. The quantification of thiocolchicoside in the samples obtained by permeation was validated with the WATERS 2487 Dual Absorbance Detector HPLC system.

2.3. Physico-chemical characterization

Fourier transform infrared (FT-IR) spectroscopy confirms the chemical characterization of two drugs developed in gel form and used as a reference, proving their similarity. The FT-IR analysis was performed using Agilent brand Digilab FTS-3500 model spectrophotometer equipped with an ATR to illuminate the structure in the wavelength spectrum range of 600-4000 cm^{-1}.

Differential scanning calorimetry (DSC) experiments were performed on two drugs developed in gel form and used as a reference. Calorimetric measurements were made using an Agilent brand DSC 200 F3 model differential scanning calorimeter. The samples were placed in tin pans and carried out in an N_2 atmosphere with a temperature increase of 10 °C min^{-1}.

2.4. In vitro permeation method

The permeation of the active ingredient thiocolchicoside in the gel form of the commercially purchased and used reference drug through the Franz Diffusion Cell was compared with this developed drug. In vitro permeation studies were performed in a Franz Diffusion Cell system with a receptor volume of 15 mL and a diffusion area of 1.77 cm^2 pH 7.4 media solution, representing the physiological region suitable for the receptor phase.

For permeability studies, three different membranes with different properties were kept in the media solution at pH 7.4, the most suitable pH for the skin, for 8 hours to saturate. Then, parameters affecting permeation, such as temperature, mixing speed, and sample amount, were changed, and the parameters that achieved the best efficiency were selected. All samples collected with the determined parameters were transferred to vials, and the amount of active ingredient was determined by the HPLC method and compared with the reference drug in the study. Permeation studies in each membrane and each parameter were performed in 3 repetitions. Thiocolchicoside permeation from a transdermal system using the Franz Diffusion Cell is shown in Figure 2.

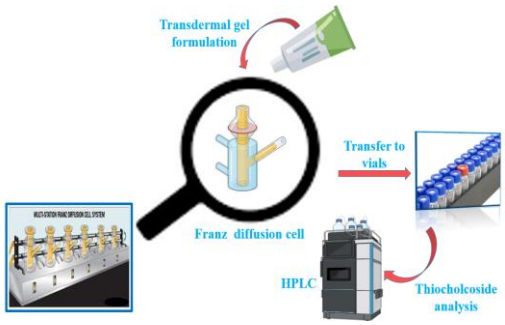

Figure 2. Thiocolchicoside permeation from a transdermal drug delivery system using the Franz Diffusion Cell

2.5. High-Performance Liquid Chromatography analysis

Waters 2487 model HPLC was used for the quantitative determination of thiocolchicoside. After filtering, standard thiocolchicoside solutions were analyzed at 280 nm with a Dual Absorbance Detector on an ACE5 C18 (4.6 mm; 150 mm; 5 µm) column. HPLC analysis method conditions were performed at 25 °C temperature, 1.2 mL/min flow rate, and 50 µL injection volume. The buffer solution contains pure water and trifluoroacetic acid. The mobile phase was prepared by adjusting acetonitrile and buffer solution in a ratio of 83:17, respectively. The dilution solution contains buffer solution and methanol in a 1:1 ratio.

3. Results and Discussion

3.1. FT-IR and DSC analysis

FT-IR spectroscopy is critical to determining the chemical interactions between thiocolchicoside and excipients. The absorption bands seen in the FT-IR spectrum give us information about the functional groups present in the structure. FT-IR spectra of the reference and developed gel drug are shown in Figure 3. When the FT-IR spectrum was analyzed, all bands were present in the developed gel drug sample. In the spectrum, the fingerprint identification peaks of the functional groups that should be present in the structure of the gel drug are seen. These peaks can be observed in all characteristic interaction spectra. The characteristic fingerprint peaks at 1647 and 1525 cm^{-1} in the FT-IR spectrum correspond to C=O and amide groups in the tropane ring. In addition, the broadening band corresponding to the O-H stretching mode is observed between 3200 and 3600 cm^{-1}. Moreover, the peak at 2953 cm^{-1} indicates the presence of C-H groups in the structure.

DSC analysis provides information on endothermic/exothermic peaks, melting, release, and compatibility between thiocolchicoside and the drug, thus providing rapid evidence. Figure 4 shows the DSC thermograms of the developed gel drug and the reference drug. The DSC thermograms show both the absence of a new peak and the presence of a characteristic endothermic peak. This peak indicates no incompatibility between the active ingredient thiocolchicoside and the drug content. An endothermic peak with a maximum of 144.3 °C in the DSC thermograms is due to melting. When the analysis results are analyzed, it is seen that the thermograms of the developed gel drug and the reference drug are compatible [45-47].

3.2. HPLC method validation

This study developed an analytical method for in vitro permeation studies of a commercial reference drug and a gel containing the active ingredient thiocolchicoside. The validation parameters of the developed method are given in Table 1. Linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ) parameters for the chromatographic method were validated. The results of the analysis will be determined by taking into account the ICH validation of analytical procedures: text and methodology Q2(R1) criteria [48-50]. For the linearity study, samples were prepared at six levels of thiocolchicoside concentration ranging from 0.078 to 40.0 µg/mL. They were evaluated by linear regression analysis in line with the obtained areas. The regression equation and correlation coefficient are y=56699572.93x+4514.39 and 0.9992, respectively.

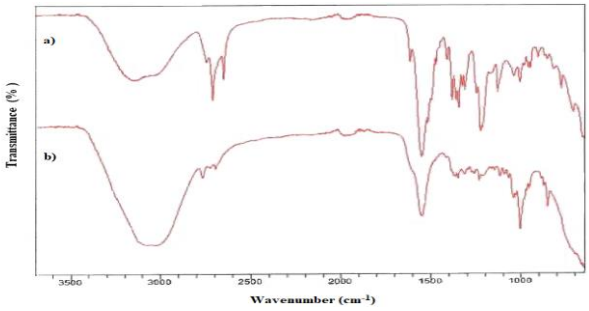

Figure 3. FT-IR spectra of a) reference drug and b) developed drug sample

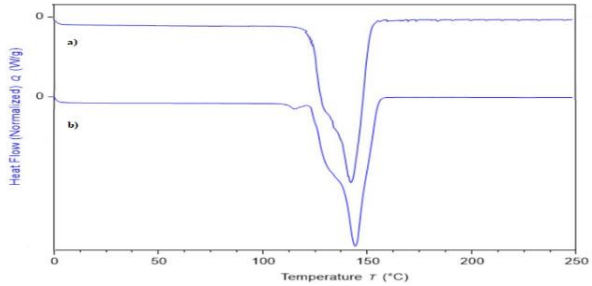

Figure 4. DSC thermograms of a) reference drug and b) developed drug sample

For the precision of this analytical method, the relative standard deviation (%RSD) value between the results of 5 different sample solutions prepared at the same concentration was determined to be less than 3%. To prove the accuracy of the analytical method, the % recovery values of the sample solutions prepared at four different concentrations in the linear region were calculated. The concentration of the value determined as 100% in accuracy and recovery studies is 26 µg/mL. Other concentration values were determined by proportioning the percentages of this value. The data obtained from 100%-102% proves the method's accuracy. Signal/noise (S/N) was used to determine LOD and LOQ values. The

signal/noise ratio determines it by comparing the result of the thiocolchicoside containing the lowest concentration of analyte with the result of the blank solution. S/N refers to 3 times the LOD and ten times the LOQ of the noise. LOD and LOQ values were calculated as 0.026 µg/mL and 0.078 µg/mL.

Table 1. Validation parameters of the developed method

Validation parameters	Level	Results		Level	Results
Accuracy	50%	101.2±0.3%	**Recovery**	30%	98.9± 0.3%
	80%	101.8±0.5%		80%	99.9± 0.1%
	100%	101.1±0.9%		100%	100.1± 0.1%
	120%	100.4±0.6%		120%	100.2± 0.2%
	150%	100.2±0.5%	**Repeatability**	100%	100.1± 0.7%

3.3. Effect of membranes on Thiocolchicoside permeation

Three different membranes with different properties specified in "Section 2.1. Reagents and chemicals" were used. It was determined how similar results were obtained with the reference drug by using a pH 7.4 media solution, 600 rpm mixing speed, 32.0 ºC temperature, and 400 mg thiocolchicoside as optimum parameters in the in vitro permeability of these membranes. When Figure 5 is examined, when the permeability of thiocolchicoside in the drug whose formulation was developed from Cellulose, Tuffryn, and Supor membranes is compared with the reference drug, the permeability differences between the membranes are seen. It can be seen from the figure that the best permeability was achieved when the Supor membrane was used, so the study continued using the Supor membrane.

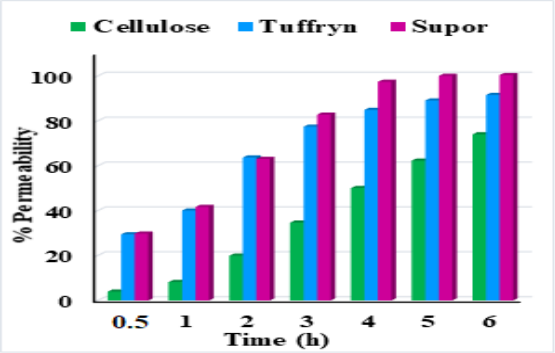

Figure 5. Effect of different membranes on thiocolchicoside permeation (Optimization conditions: solution pH: 7.4, mixing speed: 600 rpm, mixing time: 6 hours, temperature: 32.0 ºC, thiocolchicoside amount: 400 mg)

3.4. Effect of membrane saturation on Thiocolchicoside permeation

One of the most critical parameters in Franz cell studies is saturation of the membrane with sufficient media solution. After the membrane selection is determined as "Supor", the similarity of the membrane with the reference drug, both with and without saturation with the ambient solution under the selected optimum conditions, is shown in Figure 6.

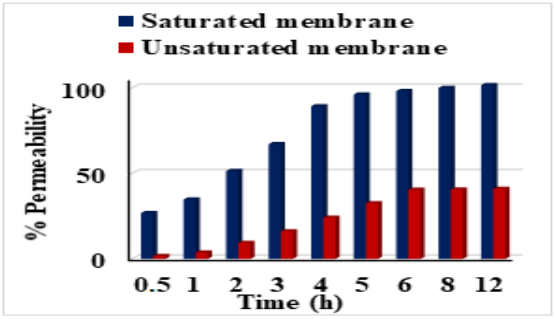

Figure 6. Effect of saturated and unsaturated membranes on thiocolchicoside permeation. (Optimization conditions: Supor membrane, solution pH: 7.4, mixing speed: 600 rpm, mixing time: 6 hours, temperature: 32.0 ºC, thiocolchicoside amount: 400 mg)

It can be seen in Figure 6 that the saturated membrane is more similar to the reference drug. The study conducted with a saturated membrane showed that 6 hours of saturation time was sufficient. For this reason, the membrane saturation time in the study was determined as 6 hours.

3.5. Effect of sample amount on Thiocolchicoside permeation

Another parameter whose effect is examined is the amount of sample placed in the Franz Diffusion Cell. Sample amounts ranging from 300 to 500 mg were inducted into the cell, questioning whether it affected thiocolchicoside permeation. A minimum of 300 mg of gel drug is required to completely cover the area of the membrane placed on the Franz Diffusion Cell and to spread the drug evenly on the membrane [51]. Examining this parameter determined that the amount of sample placed in the Franz Diffusion Cell had no effect (Figure 7). Therefore, a 400 mg thiocolchicoside sample was selected and used as an optimum sample amount in the study.

3.6. Effect of mixing time on Thiocolchicoside permeation

The amount to which the mixing time affected the permeability of thiocolchicoside in the Franz Diffusion Cell was evaluated to achieve the best resemblance with the reference drug. Under the identified ideal conditions, the amount of thiocolchicoside was measured various times, ranging from 0.5 to 8 hours, and its resemblance to the reference drug was ascertained.

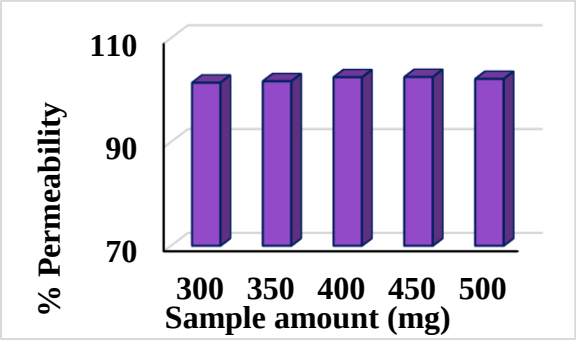

Figure 7. Effect of sample amount on thiocolchicoside permeation. (Optimization conditions: solution pH: 7.4, mixing speed: 600 rpm, mixing time: 6 hours, temperature: 32.0 ºC)

As seen in Figure 8, the amount of thiocolchicoside passing from the Franz Diffusion Cell to the environment did not vary in 6 hours or longer. Since it was similar to the reference drug, 6 hours was sufficient for the study's mixing time. The mixing time was determined to be 6 hours.

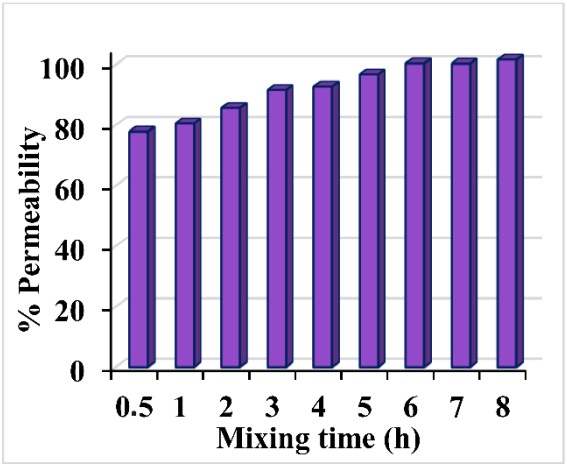

Figure 8. Effect of mixing time on thiocolchicoside permeation. (Optimization conditions: solution pH: 7.4, mixing speed: 600 rpm, temperature: 32.0 ºC, and thiocolchicoside amount: 400 mg)

3.7. Effect of mixing speed on Thiocolchicoside permeation

After determining the mixing time, another parameter is to examine the effect of mixing speed. For this parameter, the change in the permeability of thiocolchicoside was investigated by changing the mixing speed between 500 and 660 rpm. When Figure 9 is reviewed, it is seen that the similarity with the reference drug reaches 100% when mixing at 600 rpm. For this reason, 600 rpm was preferred as the working mixing speed.

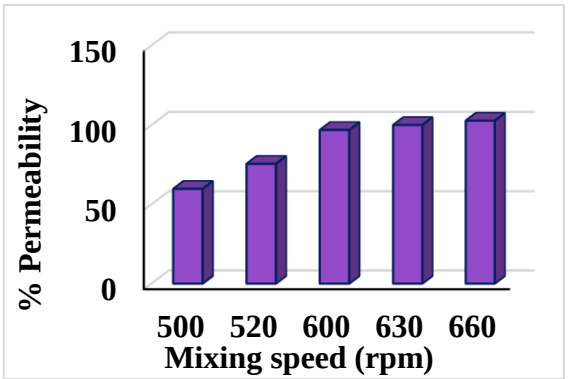

Figure 9. Effect of mixing speed on thiocolchicoside permeation. (Optimization conditions: solution pH: 7.4, mixing time: 6 hours, temperature: 32.0 ºC, and thiocolchicoside amount: 400 mg)

3.8. Effect of temperature on Thiocolchicoside permeation

In the study examining the effect of temperature on the permeation of thiocolchicoside, the

required temperature was 32.0 °C, which is the body temperature. The experiment on permeation rate is conducted at 32 ± 1 °C, except for vaginal drug products, which require a temperature of 37 ± 1 °C [52]. The permeation of thiocolchicoside was carried out at different temperatures by varying the temperatures between 30.0 and 36.0 °C. Figure 10 shows that the best permeability to the reference drug was obtained at 32.0 °C body temperature. For this reason, 32.0 °C was determined as the optimum temperature and used in the study. The temperature optimization study is to observe the permeation of thiocolchicoside at temperatures other than 32.0 °C and prove that 32.0 °C has the highest permeation.

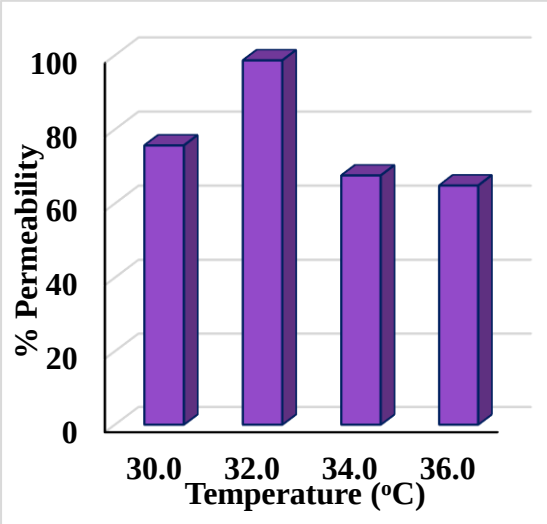

Figure 10. Effect of temperature on thiocolchicoside permeation. (Optimization conditions: solution pH: 7.4, mixing speed: 600 rpm, mixing time: 6 hours, and thiocolchicoside amount: 400 mg)

3.9. Comparison of the method with other studies

When the results obtained from the literature data are examined, it is seen that the Franz Diffusion Cell is frequently used to investigate the use of transdermal drug carrier systems and it is one of the important methods due to its originality. In a study reported by Shiow-Fern Ng and colleagues, Franz Diffusion Cells were used and their effect on the applied method was examined. For this purpose, they used ibuprofen as a model drug and synthetic membranes as a barrier. When they examined the study results, they found that it positively affected the validation [53].

In another study, Alice Simon et al. used different synthetic polymeric membranes and pig ear skin and evaluated the rivastigmine transdermal drug delivery system by performing in vitro permeation experiments in Franz Diffusion Cells. They performed a series of studies to identify the best-performing model membranes [30]. In the study examining the passage of niacinamide from different formulations into human skin, traditional Franz Diffusion Cells were used and investigated both in vitro and in vivo using a quantitative Confocal Raman Spectroscopy method including finite dose conditions. They emphasized that it is a valuable study for both actives and excipients [54].

4. Conclusions

One of the increasingly essential systems in the pharmaceutical industry is the transdermal drug delivery system, which constitutes an innovative and successful research area. In this study, the permeation of thiocolchicoside, the active ingredient taken into the body through various drug applications, with Franz Diffusion Cell, the permeability of the drug developed in gel form, and the reference drug was evaluated comparatively using the "Supor" membrane. Validation studies were carried out to evaluate the accuracy and reliability of the results obtained from the samples. In addition, although there are different studies with the thiocolchicoside agent, it will be the first comparative study with the reference drug gel. As a result of the injection of sample and reference solutions, it was determined that the linearity of the chromatograms obtained for thiocolchicoside with a correlation coefficient of 0.9992 was satisfactory for the established method.

Moreover, it was observed that the detection limit was 0.026 µg/mL, the relative standard deviation values were less than 3%, and the accuracy values were between 100% and 102%. These values obtained prove the accuracy and reliability of the study. In addition, the method is environmentally friendly in that it can be applied rapidly because it does not require many process steps, consumes few materials, and requires no toxic solutions.

Article Information Form

Acknowledgments
The authors wants to thank Düzce University.

Funding
This research was supported by the Duzce University Scientific Research Projects Fund (Project No: 2023.05.03.1406) for contributing to the financial portion of the project.

Authors' Contribution
Hale Karagüzel Investigation, Methodology; **Aslıhan Dalmaz** Methodology, Writing-Writing-review & editing; **Sezen Sivrikaya Özak** Writing-review & editing, Supervision. All authors have read and agreed to the published version of the manuscript.

The Declaration of Conflict of Interest/ Common Interest
No conflict of interest or common interest has been declared by the authors.

The Declaration of Ethics Committee Approval
This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics
The authors of the paper declare that they comply with the scientific, ethical, and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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